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Phoenix dactylifera L. seeds: a by-product as a source of bioactive compounds with antioxidant and enzyme inhibitory properties†

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Date (*Phoenix dactylifera* L.) seeds are a valuable and abundant by-product with various potential food applications and a source of functional and bioactive ingredients. In this study, date seeds from eight cultivars (Ourous "OUR", Tazizaout "TAZ", Tazarzeit "TAR", Tazoughart "TAG", Ouaouchet "OUC", Oukasaba "OUK", Delat "DEL" and Tamezwertn'telet "TWT") cultivated in the M'zab oasis (south of Algeria) were analyzed for their chemical and phytochemical compositions, antioxidant capacities and *in vitro* inhibition of some enzymes. Variations in chemical compositions were observed in the studied date seeds. The greatest contents of total phenolic compounds (476 mg GAE per g dw), total flavonoids (6.52 mg QE per g dw), anthocyanins (1.26 mg Q3GE per g dw), flavonols (3.36 mg Q3GE per g dw), proanthocyanidins (85.13 mg CE per g dw), and ascorbic acid were detected in the seeds of the TAG cultivar. All extracts manifested good antioxidant activities tested by ORAC and FRAP assays. The OUC and OUR extracts displayed the most potent antioxidant capacity against DPPH$^{\bullet}$ free radicals (IC_{50} = 37.30 μg ml^{-1}) and $ABTS^{\bullet+}$ cation radicals (IC_{50} = 13.89 μg ml^{-1}), respectively. The antioxidant activity evaluated through a xanthine/xanthine oxidase system demonstrated that the TAZ extract was more efficient as a superoxide radical scavenger (IC_{50} = 9.08 μg ml^{-1}). Date seed extracts (DSE) exhibited inhibitory activities on enzymes, showing substantial potential as skin-whitening, neuroprotective, anti-hyperglycemic or anti-hyperlipidemic agents; the inhibitory potential was tested using tyrosinase (TYR), acetylcholinesterase (AChE), α-glucosidase (α-GLU) and lipase. All date seed cultivars were able to inhibit tyrosinase and α-glucosidase in a dose-dependent manner reaching the maximum inhibition.

1. Introduction

The importance of nutraceuticals and bioactive compounds has been well recognized in connection with health promotion, disease risk reduction and health care cost reduction.[1] However, there has been growing interest in the research and development of phytochemicals such as dietary fiber, natural antioxidants and phenolic compounds from various natural sources, which could be used as functional food ingredients, nutraceuticals, or medicinal products.[2,3]

The interest in antioxidant compounds such as phenolic acids, carotenoids, vitamins and flavonoids, which are known as natural antioxidants, is largely related to their functional properties and their abilities to interfere with the formation and propagation of free radicals and protect low density lipoproteins from oxidation.[4]

Numerous epidemiological studies have consistently shown that high fruit and vegetable consumption is associated with a reduced risk of several chronic diseases, such as cardiovascular disease, neurodegenerative diseases and inflammation, as well as aging. This is attributed to the fact that these foods may provide an optimal mixture of phytochemicals.[5,6]

The date palm (*Phoenix dactylifera* L.) is one of the major fruit crops produced in arid and semiarid regions of the world such as North Africa and the Middle East.[2,7] The fruit is composed of a fleshy pericarp and seed which constitute 10 to 15% of the date's weight. In addition to its dietary use, the dates are of medicinal use and are used to treat a variety of ailments in the various traditional systems of medicine.

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Preclinical studies have shown that date fruits exhibit free radical scavenging, antioxidant, antimutagenic, antimicrobial, anti-inflammatory, gastroprotective, hepatoprotective, nephroprotective, anticancer and immunostimulant activities.[8,9]

The beneficial effects of dates are not limited to the date fruit only but date seeds or pits, which are a by-product of many industries based on the technological transformation of the date fruits or their biological transformation (date powders, pitted dates, date syrup, date confectionery, date juice, *etc*.).[10,11]

Date seeds (*Phoenix dactylifera* L.) may have extractable high-value-added components. However very little uses are known for this by-product, rather, they are discarded or generally consumed either as a conventional soil fertilizer or as complementary feed materials for animals and poultry. Also, they have been used to make coffee for people preferring a non-caffeinated coffee.[12–14] The approximate annual yield of date seeds, in most producing countries, ranges from 1000 to 10 000 tons. Thus, utilization of such waste is very important for date cultivation and to increase the income to this sector.[15,16] Due to their interesting properties and compositions, date seed extracts could represent a source of bioactive substances for human health.[17–19]

Nevertheless, limited research has been conducted on the valorization of this abundant by-product for the development of pharmaceutical or nutraceutical products. These seeds are generally discarded as waste but their significance as a bioactive product is highlighted here.

Therefore, the first aim of this investigation was to evaluate the chemical composition and nutritional value of eight different cultivars of date seeds (Ourous "OUR", Tazizaout "TAZ", Tazarzeit "TAR", Tazoughart "TAG", Ouaouchet "OUC", Oukasaba "OUK", Delat "DEL" and Tamezwertn'telet "TWT"), their phytochemical contents and antioxidant properties. Seeds in general are the most important component of a fruit from the survival perspective of a plant, which might explain the exceptionally high concentration of secondary metabolites, capable of interacting with reactive oxygen species and preventing their damage, which is related to neurodegenerative and metabolic diseases. For these reasons, the second aim of this work was to test the potential of date seeds as antidiabetic and anti-obesity substances using the enzymatic inhibition of α-glucosidase (α-GLU) and lipase; the neuroprotective potential was measured as the inhibition of acetylcholinesterase (AChE). Finally, tyrosinase inhibition was tested as a means of measuring potential cosmetic applications, as the enzyme is implicated in human skin hyperpigmentation.

2. Materials and methods

2.1. Chemicals

The Folin–Ciocalteu reagent, aluminium chloride (≥97.0% purity) and potassium persulfate (≥99.99% purity) were from Biochem, Chemopharma (Montreal, Quebec); sodium carbonate anhydrous, sodium hydrogen phosphate and ascorbic acid (≥99.53 purity) were from Biochem, Chemopharma (Georgia, USA); quercetin was from Panpharma (Shanghai, China); ferric chloride was from Panreac (Barcelona, Spain); 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-dichloroindophenol sodium salt hydrate (DCPIP), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) were from Sigma Chemical (Sigma–Aldrich GmbH, Germany); gallic acid was from Prolabo (Montreuil, France); β-carotene was from Fluka Biochemika (Steinheim, Germany); and acetone, butanol, ethanol and methanol (99.8% purity) were from VWR, Prolabo (CE-EMB).

Nitrotetrazolium blue chloride (NBT), acetylthiocholine iodide (ATCI), DTNB, Tris, dimethyl sulfoxide (DMSO), galantamine, 3,4-dihydroxy-L-phenylalanine (L-DOPA), tyrosinase, α-kojic, α-glucosidase, acarbose, and 2-nitrophenyl β-D-glucopyranoside (pNPG) were acquired through Sigma-Aldrich (Madrid, Spain).

2.2. Plant materials and extraction procedure

Date palm fruits were obtained from the M'zab oasis of the Ghardaia region in southern Algeria. The seeds of eight different cultivars locally known as Ourous (OUR), Tazizaout (TAZ), Tazarzeit (TAR), Tazoughart (TAG), Ouaouchet (OUC), Oukasaba (OUK), Delat (DEL) and Tamezwertn'telet (TWT) were directly isolated from date fruits collected at the mature stage.

Removed seeds were soaked in water, washed to free them from any adhering date flesh and then dried at 50 °C for 24 h. The dried seeds of each cultivar were separately ground into a fine powder (250 μm of diameter) with a coffee grinder after having crushed them to small pieces using a mortar. The extracts were prepared from each sample by continuous agitation. Briefly, the powder was extracted three times at room temperature for 24 h, in acetone/H_2O (75/25, v/v). The mixture was centrifuged and then filtered and the obtained filtrates were evaporated in a rotary evaporator (Rotavapor R-200/205, Buchi, Flawil, Switzerland) at 40 °C. The aqueous extracts were frozen at −20 °C in a freezer for 24 h and finally lyophilized using a Christ Alpha 1–4 LD freeze-dryer over 10 days. A powdery residue was obtained and stored at −20 °C in an airtight container until use. The yield of extraction was estimated for each date seed cultivar.

2.3. Proximate analysis and chemical composition

Date seeds were analyzed for their chemical composition (ash, moisture, carbohydrates, protein, proline and free amino acid "FAA"). The ash content was determined by incineration at 550 ± 15 °C for 3 h; the moisture content was estimated by oven drying the sample to a constant weight (24 h, 105 °C). The procedure reported by Dubois *et al*.[20] was carried out to determine the total carbohydrate composition. The crude protein content was determined according to the Bradford[21] method; the proline content was determined by the spectrophotometric method described by Bates *et al.*[22] The FAA content was determined using the method of Yemm and Cocking.[23]

2.4. Phytochemical analysis of date seed extracts

2.4.1. Total phenolic (TPC) and flavonoid contents. The total phenolic content was evaluated using the Folin–Ciocalteu reagent according to Al-Farsi *et al.*[24] A volume of 1 : 10 diluted Folin–Ciocalteu reagent (0.75 ml) was added to 0.1 ml of the extract. After 4 min, 0.75 ml of sodium carbonate (6%) was added. Each tube content was thoroughly mixed and then incubated at room temperature for 1 h. The absorbance was read at 760 nm using a spectrophotometer (Uviline 9400, Secomam, France). Gallic acid was used for the standard calibration curve. Results were expressed as mg of gallic acid equivalents (GAE) per g dry weight (dw).

The total flavonoid content was determined by the assay reported by Quettier-Deleu *et al.*[25] In each tube, 0.75 ml of a 2% $AlCl_3$ solution was added to equal volumes of extracts. The mixture was incubated for 15 min and then the absorbance was read at 410 nm. The flavonoid content was expressed as mg of quercetin equivalents (QE) per g dw.

2.4.2. Anthocyanin and flavonol contents. Anthocyanins and flavonols were extracted according to the procedure described by Yoshitama *et al.*[26] Briefly, one gram of date seed powder was homogenized with 10 ml of acidified methanol (methanol/0.1 N HCl). After overnight incubation, 900 μL of the supernatant was added to 900 μL methanol/0.1 N HCl. The anthocyanin concentration was determined from the absorbance at 530 nm using a molar extinction coefficient (ε) of 38 000 L mol^{-1} cm^{-1}. The content of flavonols was determined at 360 nm (ε = 20 000 L mol^{-1} cm^{-1}). Anthocyanin (AC) and flavonol contents (FC) were expressed as mg of quercetin 3-glucoside equivalents (Q3GE) per g dw.

2.4.3. Proanthocyanidin (PA) content. The proanthocyanidin contents were determined according to Škerget *et al.*[27] The extract (200 μL) was mixed with 3 ml of reagent (HCl–butanol + $FeSO_4$) and then incubated at 95 °C in a water-bath for 15 min. The absorbance was determined at 530 nm. The results were expressed as mg cyanidin equivalents (CE) per g dw, using a molar extinction coefficient of cyanidins (ε = 34 700 L mol^{-1} cm^{-1}).

2.4.4. Determination of the ascorbic acid content (AAC). The determination of the ascorbic acid content was carried out using a method described by Klein and Perry.[28] A weight portion of each cultivar (1.5 g) was mixed with 15 ml of oxalic acid (3%) for 40 min. After centrifugation, one hundred microlitres of the supernatant were mixed with 1 ml of DCPIP and the absorbance was measured at 515 nm. The ascorbic acid content was expressed as mg ascorbic acid equivalents (AAE) per g dry dw.

2.4.5. HPLC analysis of phenolics. HPLC analysis was carried out using a Jasco HPLC system (Tokyo, Japan) consisting of a Jasco RHPLC pump (PU-4180), a Jasco Photo Diode Array Detector (MD-4010), a Jasco Intelligent Sampler (AS-2055 Plus), a Jasco Column Oven (CO-4061), a Jasco Bottle Stand (BS-4000-1), a Jasco LC-Net II/ADC interface box and Chrom NAV Control Center software attached to a computer system. Analytical separation of phenolic compounds was carried out on a Kinetex column C18 (250 × 4.6 mm; 5 μm) (Phenomenex, CA, USA). After filtration using Millipore filters (0.45 μm), 20 μl of each extract dissolved in 99 : 01 methanol : HCOOH was injected at a concentration of 5 mg ml^{-1}. Samples were run at a flow rate of 1 ml min^{-1} and a gradient system elution of redistilled water : formic acid in a ratio of 98 : 2 (mobile phase "A") and redistilled water : acetonitrile : formic acid in a ratio of 49.75 : 49.75 : 0.5 ("B"). The solvent gradient was programmed as follows: 10% B at 0 min, increasing from 0–50 min to 55%, 50–65 min 55–95% B, 65–70 min 10% B. The corresponding chromatograms of the phenolics were recorded at wavelengths of 280 nm and 360 nm. Identification of phenolic compounds was confirmed by comparing their retention time and the retention time of the phenolic standard. The peak areas of standard curves of the corresponding phenolics at wavelengths of 280 and 360 nm were used for calculations of phenolics.

2.5. *In vitro* antioxidant activity

2.5.1. Oxygen radical antioxidant capacity (ORAC) method. The capacity of date seed extracts to scavenge peroxyl radicals was determined using the ORAC method. This assay was carried out according to the method used by Dávalos, Gómez-Cordovés and Bartolomé.[29] Lyophilized samples were dissolved in methanol at a concentration of 1 mg ml^{-1} and then diluted so that the concentrations in the wells ranged from 1 to 500 μg ml^{-1}. Different concentrations of each sample were incubated with fluorescein (70 nM) for 10 min at 37 °C in 96-well plates. After incubation, AAPH (12 mM) was added and the fluorescence was recorded for 98 min at excitation and emission wavelengths of 485 nm and 520 nm, respectively, in a FLUO star Optima fluorimeter (BMG Labtech, Ortenberg, Germany). Results were expressed as mmol Trolox equivalents (T eq.) per g dw.

2.5.2. Ferric reducing antioxidant potential (FRAP). The procedure described by Benzie and Strain[30] was used. The FRAP reagent containing TPTZ solution (10 mM 2,4,6-tris (2-pyridyl)-1,3,5-triazine in 40 mM HCl), $FeCl_3$ solution (5 mM) and sodium acetate buffer (300 mM, pH 3.6) was freshly prepared in proportions of 1 : 1 : 10 (v/v/v). Briefly, an aliquot of the extract (100 μL) was mixed thoroughly with 900 μL of the FRAP reagent. After incubation for 30 min at 37 °C, the absorbance of the mixture was measured at 593 nm. The absorbance was compared to a $FeSO_4$ standard curve and the FRAP values were expressed as mmol of Fe^{2+} equivalents (Fe^{2+} eq.) per g dw.

2.5.3. DPPH$^{\bullet}$ radical scavenging assay. The DPPH$^{\bullet}$ radical scavenging assay is employed as previously described.[31] Briefly, 100 μL of the extract was mixed with 1 ml of 60 μM DPPH$^{\bullet}$. The test tubes were incubated in the dark for 30 min before reading the absorbance at 517 nm. Trolox was used as a standard and the free scavenging activity was expressed as mmol Trolox equivalents (mmol T eq.) per g dw. Results were also presented as IC_{50}, the extract concentration required to scavenge 50% of the DPPH$^{\bullet}$.

2.5.4. ABTS$^{\bullet+}$ scavenging assay. This assay is based on the ABTS$^{\bullet+}$ radical cation discoloration used according to the

method of Re *et al.*[32] The working solution was prepared by mixing the ABTS salt dissolved in deionized water to a concentration of 7 mM with potassium persulfate added to a final concentration of 2.45 mM. The obtained solution was then diluted with methanol until an absorbance of 0.70 ± 0.02 at 734 nm was achieved. The extract was mixed with 1 ml of diluted $ABTS^{\bullet+}$ solution and incubated at 30 °C. After 6 min, the absorbance was measured at 734 nm. The results were expressed as mmol T eq. per g dw. The IC_{50} of the $ABTS^{\bullet+}$ radical cation was determined for each extract.

2.5.5. Superoxide radical scavenging activity. The xanthine/xanthine oxidase assay was employed in order to assess the capacity of date seed extracts (DSE) to scavenge superoxide radicals generated by the reaction.[33] The assay was performed in 96-well microplates, and the mixture contained 90 μM xanthine, 16 mM Na_2CO_3 and 22.8 μM NBT dissolved in phosphate buffer (pH 7). Then, 30 μL of the sample at different concentrations and 30 μL of xanthine oxidase (168 U l^{-1}) were added to start the reaction. The mixture was incubated for 2 min at 37 °C. The absorbance was measured at 560 nm and the activity was determined by measuring the transformation of NBT to the blue chromogen formazan by the superoxide radical ($O_2^{\bullet-}$). Gallic acid was used as the reference. The results were expressed as %, as calculated with the following equation:

$$(\%) = [(\mathrm{Abs}_{\mathrm{control}} - \mathrm{Abs}_{\mathrm{sample}})/\mathrm{Abs}_{\mathrm{control}}] \times 100. \quad (1)$$

2.6. *In vitro* bioactive properties of *P. dactylifera* seed extracts against physiologically relevant enzymes

2.6.1. Xanthine oxidase (XO) inhibition. The effect of the DSE on xanthine oxidase was evaluated by measuring the formation of uric acid from xanthine at 295 nm after 2 min. The wells contained the same components as described above in the xanthine/xanthine oxidase system but the reaction mixture did not contain NBT.

2.6.2. Tyrosinase (TYR) inhibition. The inhibition of tyrosinase was performed in a 96-well plate using the modified dopachrome method with L-DOPA as the substrate.[34] Each well contained a mix of 10 μL of different sample concentrations in DMSO, 40 μL of L-DOPA, 80 μL of phosphate buffer (75 mM, pH 6.8) and 40 μL of tyrosinase (TYR). In this assay α-kojic acid was used as a reference inhibitor. The absorbance was measured at 475 nm. Tyrosinase inhibition was expressed as a percentage (1).

2.6.3. α-Glucosidase (α-GLU) inhibition. The capacity of date seed extracts to inhibit α-glucosidase of *Saccharomyces cerevisiae* was assessed in 96-well microplates at 405 nm following the procedure reported by Kazeem *et al.*[35] The mixture used for the assay contained 50 μL of different sample concentrations, acarbose (reference compound) and 100 μL of α-glucosidase enzyme (1 U ml^{-1}). After 10 min of pre-incubation, 50 μL of pNPG (3 mM) dissolved in phosphate buffer (20 mM, pH 6.9) were added and incubated at 37 °C for 20 min. The blank contained buffer instead of α-glucosidase and the results were expressed as percentages.

2.6.4. Acetylcholinesterase (AChE) inhibition. AChE inhibition was measured by Ellman's method applied to 96-well microplates.[36] Each well contained 25 μL of ATCI (15 mM) in ultrapure water, 125 μL of DTNB (3 mM) in buffer C (50 mM Tris–HCl, pH 8; 100 mM NaCl and 20 mM $MgCl_2{\cdot}6H_2O$), 50 μL of buffer B (50 mM Tris–HCl, pH 8 and 0.1% bovine serum) and 25 μL of different sample concentrations in buffer A (50 mM Tris–HCl, pH 8). Galantamine was used as a positive inhibitor. Finally, 25 μL of the enzyme (0.22 U ml^{-1}) was added to complete the reaction. The absorbance was measured eight times over 11 min at 405 nm.

2.6.5. Lipase inhibition. The lipase inhibition was evaluated according to the method of Spínola *et al.*[37] using a 96-microplate reader. Each well contained 40 μL of the tested sample and 40 μL of lipase type II from porcine pancreas (2.5 mg ml^{-1} prepared in Tris-Buffer (100 mM Tris–HCl and 5 mM $CaCl_2$, pH 7)). After preincubation for 15 min, 20 μL of 10 mM pNPB solution was added to each well. After incubation for another 15 min at 37 °C, the absorbance was read at 405 nm. Orlistat was used as a reference inhibitor. The inhibitory activity was expressed as a percentage (1).

2.7. Cytotoxicity screening in fibroblasts (3T3-L1 cell line)

DSE antiproliferative effects in 3T3-L1 murine pre-adipocytes (CL-173; American Type Culture Collection, USA) were assessed using a mitochondrial viability test (MTT assay).[38] 3T3-L1 cells were grown in cell culture flasks in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum and 1% penicillin streptomycin–glutamine. Cultures were incubated in the presence of 5% CO_2 at 37 °C, under 100% relative humidity. Cells were seeded in 96-well microplates at a density of 5×10^3 cells per well and grown for 24 h at 37 °C. They were then treated with various concentrations of seed extracts (0.016, 0.031, 0.063, 0.125, 0.250, 0.500 and 1 mg ml^{-1}) for 24 h, and non-treated cells as controls. Then, 100 μL of new medium with the MTT reagent (0.4 mg ml^{-1} final concentration) was added to each well, before incubation for 3 h at 37 °C. The MTT containing media were then removed and the formazan crystals were dissolved in DMSO. Cell survival was measured as the reduction of MTT into formazan at 550 nm, using the microplate reader.

2.8. Statistical analysis

All tests were carried out in triplicate at different days, with each experiment run at least three times. Data represent means ± SEM for the total number of experiments done. GraphPad Prism v.6 was required to perform data analyses and nonlinear regression analysis, and obtaining figures and statistics. Data distribution was established following a one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons with a confidence interval of 95% ($p < 0.05$).

3. Results

3.1. Proximate analysis and chemical composition

The evaluation of the chemical composition obtained for *P. dactylifera* date seeds of the different cultivars is shown in Table 1. The percentage of ash and moisture ranged between 11 to 12% and 10.8 to 11.4%, respectively. Carbohydrates were found to range from 17.4 to 27.8 g glucose equivalents per 100 g dw, meanwhile protein was 1.4 to 3.3 g BSA equivalents per 100 g dw. The analysis of proline and FAA contents from different cultivars showed large variations; the TAG and TAZ cultivars contained relatively high contents with 38.7 mg proline equivalents per 100 g dw and 404.3 mg leucine equivalents per 100 g dw, respectively.

3.2. Phytochemical composition of date seeds

As shown in Table 2, the evaluation of the total phenolic compound contents from the different cultivars of date seeds revealed that TAG contained significantly the highest contents of phenolic and flavonoid compounds (475.5 ± 1.32 mg GAE per g dw and 6.5 mg QE per g dw, respectively), compared with other cultivars. On the other hand, TAG gave the most important yield.

Analysis of individual phenolic compounds from DSE by HPLC-DAD-MS at 280 and 360 nm allowed the characterization of three different phenolic acids (ferulic, vanillic and syringic acids) and two flavonoids (isoquercetin and rutin) (Table 4). Ferulic and vanillic acids were the major phenolics of date seed extracts and their contents ranged from 1.104 (TAG) to 3.802 mg per g dw (TAZ) and from 0.326 (TAZ) to 0.627 mg per g dw (TAG), respectively. In the OUC cultivar no phenolic compounds were detected, despite the chromatographic profile exhibiting main peaks, and it could be due to the presence of compounds other than the studied compounds (ESI†).

The anthocyanin (AC) and flavonol (FC) contents ranged between 1.29 and 0.64 and from 3.36 to 2.11 mg Q3GE per g (dw), respectively. The analysis of proanthocyanidin (PA) and ascorbic acid (AAC) contents from different cultivars revealed large variations, and the TAG and TWT cultivars contained relatively high contents (85.1 mg CE per g dw and 0.81 mg AAE per g dw, respectively).

3.3. *In vitro* antioxidant activity

The antioxidant activities of the different DSE were evaluated using different antioxidant tests: ORAC, FRAP, DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and superoxide radicals. As shown in Table 3, all extracts displayed a good antioxidant capacity. The capacity of DSE to scavenge peroxyl radicals determined using the ORAC method was between 4.93 and 5.98 mmol T eq. per g dw. According to the results of FRAP assay, the TAZ extract displayed the highest reducing power with 3.16 mmol. Fe^{2+} eq. per g.

The antioxidant activity of DSE was also determined by following the reduction of the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals (Table 3). All tested extracts exhibited efficient DPPH$^{\bullet}$ and

Table 1 Proximate chemical compositions of the different date seed cultivars

Cultivars	Ash (%)	Moisture (%)	Total sugar (g GE per 100 g)	Protein (g BSAE per 100 g)	Proline (mg PE per 100 g)	FAA (mg LE per 100 g)
OUR	11.93 ± 0.312^{ab}	11.37 ± 0.067^{ab}	20.97 ± 0.219^{d}	1.39 ± 0.145^{d}	24.60 ± 0.887^{cd}	320.37 ± 2.871^{c}
TAZ	12.08 ± 0.232^{a}	11.36 ± 0.276^{ab}	17.36 ± 0.560^{e}	2.64 ± 0.195^{bc}	34.56 ± 0.671^{ab}	404.33 ± 1.569^{a}
TAR	11.13 ± 0.355^{bc}	10.66 ± 0.022^{d}	24.11 ± 0.512^{c}	2.83 ± 0.040^{bc}	25.81 ± 1.405^{c}	316.94 ± 4.868^{c}
TAG	11.77 ± 0.422^{abc}	11.25 ± 0.15^{abc}	25.67 ± 0.146^{b}	2.98 ± 0.056^{abc}	38.65 ± 1.006^{a}	303.99 ± 4.394^{cd}
OUC	11.01 ± 0.289^{c}	10.86 ± 0.146^{abc}	19.93 ± 0.390^{d}	2.51 ± 0.083^{c}	23.58 ± 0.426^{cd}	277.94 ± 2.047^{e}
OUK	11.58 ± 0.148^{abc}	11.43 ± 0.034^{a}	29.47 ± 0.097^{a}	3.10 ± 0.044^{ab}	31.95 ± 0.738^{b}	342.25 ± 2.617^{b}
DEL	11.87 ± 0.053^{abc}	11.09 ± 0.091^{abc}	17.61 ± 0.219^{e}	2.83 ± 0.106^{bc}	21.07 ± 1.216^{d}	340.91 ± 6.064^{b}
TWT	12.06 ± 0.030^{a}	10.80 ± 0.016^{cd}	27.78 ± 0.097^{b}	3.34 ± 0.035^{a}	14.37 ± 0.853^{e}	295.36 ± 2.974^{de}

Values marked with different letters along the same column are significantly different ($p < 0.05$). GE: glucose equivalent; BSAE: bovine serum albumin equivalent; PE: proline equivalent and LE: leucine equivalent.

Table 2 Extraction yields and antioxidant contents from seeds of *Phoenix dactylifera* L. cultivars

Cultivars	Yield (%)	TPC (mg GAE per g dw)	TFC (mg QE per g dw)	AC (mg Q3GE per g dw)	FC (mg Q3GE per g dw)	PA (mg CE per g dw)	AAC (mg AAE per g dw)
OUR	20.53 ± 0.525^{e}	342.69 ± 0.239^{f}	5.38 ± 0.031^{d}	0.99 ± 0.028^{b}	2.85 ± 0.042^{b}	49.17 ± 1.614^{c}	0.79 ± 0.046^{a}
TAZ	23.52 ± 0.554^{de}	353.67 ± 0.359^{e}	5.38 ± 0.033^{d}	0.76 ± 0.019^{d}	2.55 ± 0.49	52.47 ± 0.301^{c}	0.56 ± 0.029^{c}
TAR	35.36 ± 0.606^{b}	426.40 ± 1.196^{b}	6.03 ± 0.034^{b}	0.80 ± 0.008^{cd}	2.33 ± 0.012^{e}	74.66 ± 1.188^{b}	0.58 ± 0.007^{c}
TAG	43.04 ± 1.665^{a}	475.51 ± 1.316^{a}	6.52 ± 0.001^{a}	1.26 ± 0.016^{a}	3.36 ± 0.032^{a}	85.13 ± 1.945^{a}	0.75 ± 0.005^{ab}
OUC	24.91 ± 1.254^{cde}	383.51 ± 1.077^{c}	5.55 ± 0.063^{c}	0.97 ± 0.008^{b}	2.64 ± 0.007^{c}	64.76 ± 0.821^{b}	0.72 ± 0.012^{ab}
OUK	28.64 ± 0.881^{c}	360.92 ± 0.479^{d}	5.92 ± 0.031^{b}	0.73 ± 0.042^{de}	2.40 ± 0.092^{de}	67.60 ± 2.585^{b}	0.67 ± 0.015^{bc}
DEL	22.93 ± 0.897^{de}	327.35 ± 0.239^{h}	5.33 ± 0.002^{d}	0.64 ± 0.004^{e}	2.11 ± 0.009	53.72 ± 0.746^{c}	0.67 ± 0.009^{bc}
TWT	27.04 ± 0.462^{cd}	336.05 ± 0.479^{g}	4.41 ± 0.032^{e}	0.87 ± 0.003^{c}	2.54 ± 0.005^{cd}	59.77 ± 4.445^{b}	0.81 ± 0.034^{a}

Values marked with different letters along the same column are significantly different ($p < 0.05$). TPC: total phenolic content; TFC: total flavonoid content; AC: anthocyanin content; FC: flavonol content; PA: proanthocyanidins and AAC: ascorbic acid content.

Table 3 Antioxidant activities from seeds of *Phoenix dactylifera* L. cultivars

Cultivars	ORAC (mmol T eq. per g)	FRAP (mmol Fe^{2+} eq. per g)	$DPPH^{\bullet}$ scavenging activity		$ABTS^{\bullet+}$ scavenging activity	
			(mmol T eq. per g)	(IC_{50} μg ml^{-1})	(mmol T eq. per g)	(IC_{50} μg ml^{-1})
OUR	5.85 ± 0.526^{a}	2.96 ± 0.029^{b}	3.39 ± 0.033^{d}	55.57 ± 0.534^{c}	10.10± 0.238^{a}	13.89 ± 0.327^{d}
TAZ	5.31 ± 0.549^{a}	3.16 ± 0.021^{a}	3.59 ± 0.049^{c}	52.55 ± 0.717^{d}	5.65± 0.103^{b}	24.82 ± 0.451^{c}
TAR	4.93 ± 0.371^{a}	2.59 ± 0.002^{c}	2.74 ± 0.006^{f}	68.90 ± 0.163^{a}	5.18± 0.101^{bc}	27.09 ± 0.530^{b}
TAG	5.86 ± 0.485^{a}	2.28 ± 0.014^{d}	2.99 ± 0.008^{e}	63.16 ± 0.177^{b}	5.50± 0.058^{bc}	25.49 ± 0.270^{c}
OUC	5.02 ± 0.278^{a}	1.78 ± 0.003^{e}	5.06 ± 0.019^{a}	37.30 ± 0.144^{f}	5.10± 0.042^{c}	27.46 ± 0.227^{b}
OUK	5.57 ± 0.198^{a}	1.67 ± 0.013^{f}	4.52 ± 0.060^{b}	41.72 ± 0.556^{e}	4.52 ± 0.001^{d}	31.02 ± 0.004^{a}
DEL	4.99 ± 0.172^{a}	1.48 ± 0.004^{g}	3.42 ± 0.020^{d}	55.16 ± 0.322^{c}	4.49± 0.029^{d}	31.24 ± 0.205^{a}
TWT	5.98 ± 0.116^{a}	1.45 ± 0.002^{g}	3.64 ± 0.021^{c}	51.87 ± 0.297^{d}	4.34± 0.023^{d}	32.31 ± 0.169^{a}

Values marked with different letters along the same column are significantly different ($p < 0.05$).

Table 4 Phenolic acid and flavonoid compositions determined by HPLC-DAD-MS from seeds of *Phoenix dactylifera* L. cultivars

Cultivars	Compound (mg g^{-1})					
	Ferulic acid	Vanillic acid	Syringic acid	Isoquercetine	Rutin	Total
OUR	2.8360 ± 0.0638^{c}	0.4430 ± 0.0251^{e}	0.0459 ± 0.0014^{d}	0.0047 ± 0.0002^{b}	—	3.330
TAZ	3.8623 ± 0.2492^{a}	0.3263 ± 0.0124^{f}	0.1800 ± 0.0052^{a}	0.0110 ± 0.0016^{a}	—	4.380
TAR	1.2707 ± 0.1109^{d}	0.4906 ± 0.0319^{cd}	—	—	0.2336 ± 0.0146^{a}	1.995
TAG	1.1038 ± 0.0222^{d}	0.6256 ± 0.0166^{a}	—	—	0.2079 ± 0.0105^{b}	1.937
OUC	—	—	—	—	—	—
OUK	2.7402 ± 0.0706^{c}	0.5999 ± 0.0253^{ab}	0.0676 ± 0.0033^{b}	0.0048 ± 0.0003^{b}	—	3.312
DEL	3.8019 ± 0.2375^{a}	0.5353 ± 0.0371^{bc}	0.0567 ± 0.0018^{c}	0.0051 ± 0.0002^{b}	—	4.399
TWT	3.2910 ± 0.1581^{b}	0.4756 ± 0.0291^{cd}	0.0690 ± 0.0049^{b}	0.0058 ± 0.0001^{b}	—	3.841
Calibration curves of phenolic compounds						
Equation	$Y = 5 \times 10^{7}x -$ 10514	$Y = 6 \times 10^{6}x -$ 1281.4	$Y = 2 \times 10^{8}x -$ 4517.8	$Y = 2 \times 10^{8}x +$ 351.27	$Y = 3 \times 10^{7}x -$ 63.393	
LOD	0.0028	0.00885	0.0030082	0.000858	0.002898	
LOQ	0.009334	0.0295	0.010273	0.002861	0.009659	
Linearity range (μg ml^{-1})	0.25–12.5	1–50	0.25–12.5	0.25–12.5	0.25–12.5	

Values marked with different letters along the same column are significantly different ($p < 0.05$). LOD: limit of detection and LOQ: limit of quantification.

$ABTS^{\bullet+}$ scavenging activities in a dose dependent manner. Interestingly, OUC and OUR extracts displayed significantly stronger Trolox equivalent antioxidant capacity (TEAC) with $DPPH^{\bullet}$ (5.06 ± 0.02) and $ABTS^{\bullet+}$ (10.10 ± 0.24) radical scavenging activities, respectively. Regarding the IC_{50} values, a lower IC_{50} value indicates a higher scavenging effect.

The DSE was also able to scavenge superoxide radicals generated by the xanthine/xanthine oxidase system in a dose dependent manner as shown in Fig. 1. The antiradical activity of each extract was compared to that of gallic acid. The IC_{50} values corresponding to the amount of extract needed to scavenge 50% of the superoxide radical showed that the TAR cultivar exhibited the greatest scavenging activity (9.08 μg ml^{-1}) compared to other cultivars (Table 5).

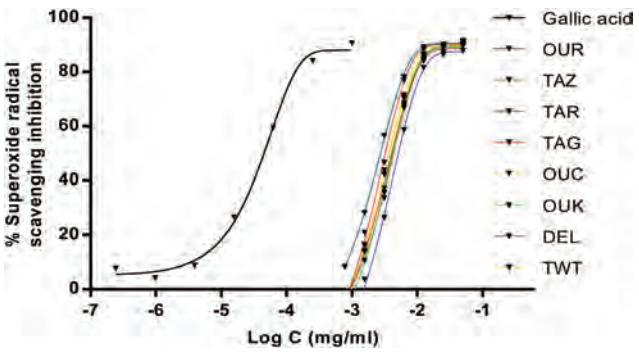

Fig. 1 Antiradical potential of DSE against superoxide radicals generated by the xanthine/xanthine oxidase method.

3.4. *In vitro* bioactive properties of *P. dactylifera* seed extracts

The date seed extracts (DSE) were found to be able to inhibit TYR and α-GLU in a dose-dependent manner from 5% at 4 μg ml^{-1} to approximately 88% at 1000 μg ml^{-1} and 11% at 2 μg ml^{-1} to 95% at 25 μg ml^{-1}, respectively (Fig. 2 and 3). The IC_{50} values corresponding to the amount of extract needed to inhibit 50% of the enzyme activity showed that the OUR cultivar exhibited the best TYR inhibition (34.46 μg ml^{-1}) compared to other cultivars (Table 5) but it was lower than that of kojic acid (0.12 μg ml^{-1}). DSE exhibited higher inhibition of

Table 5 IC_{50} values (calculated by non-linear regression) for seeds of *Phoenix dactylifera* L. extracts and the reference inhibitors used in different bioassays

	Bioassay (μg ml^{-1})				
Cultivars	TYR inhibition	α-GLU inhibition	AChE inhibition	Lipase inhibition	Superoxide radical scavenging
OUR	34.46 ± 0.130^{a}	10.46 ± 0.529^{a}	nd	nd	14.51 ± 2.868^{ab}
TAZ	60.93 ± 4.222^{bc}	13.54 ± 0.808^{b}	nd	nd	14.50 ± 3.030^{ab}
TAR	64.42 ± 13.540^{c}	10.69 ± 0.622^{a}	nd	nd	9.08 ± 3.121^{a}
TAG	65.69 ± 9.232^{c}	10.31 ± 0.748^{a}	nd	nd	12.98 ± 2.096^{ab}
OUC	48.25 ± 5.201^{abc}	11.48 ± 0.847^{a}	nd	nd	13.67 ± 2.527^{ab}
OUK	45.40 ± 1.911^{ab}	11.74 ± 0.572^{ab}	nd	nd	15.35 ± 1.701^{ab}
DEL	58.40 ± 3.716^{bc}	12.09 ± 0.856^{ab}	nd	nd	18.22 ± 1.573^{b}
TWT	55.24 ± 1.631^{bc}	10.69 ± 0.581^{a}	nd	nd	13.22 ± 0.5556^{ab}
Reference inhibitors	Kojic acid	Acarbose	Galantamine	Orlistat	Gallic acid
	0.12 ± 0.011	369.38 ± 49.070	0.68 ± 0.064	5.63 ± 2.718	0.05 ± 0.011

Values marked with different letters along the same column are significantly different ($p < 0.05$). nd: not determined (the IC_{50} values were found to be greater than 1000 μg ml^{-1}).

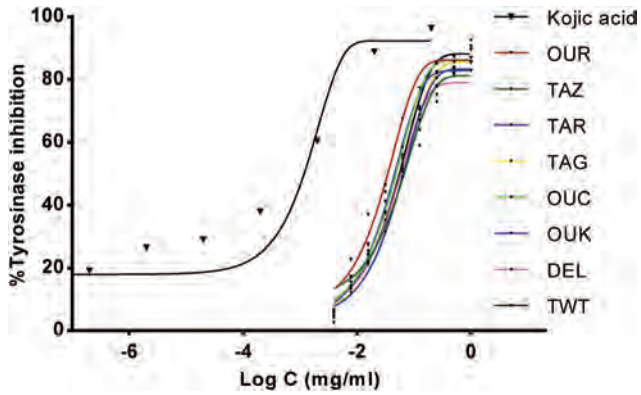

Fig. 2 Bioassays for determining anti-tyrosinase potential of date seed extracts (DSE) and kojic acid.

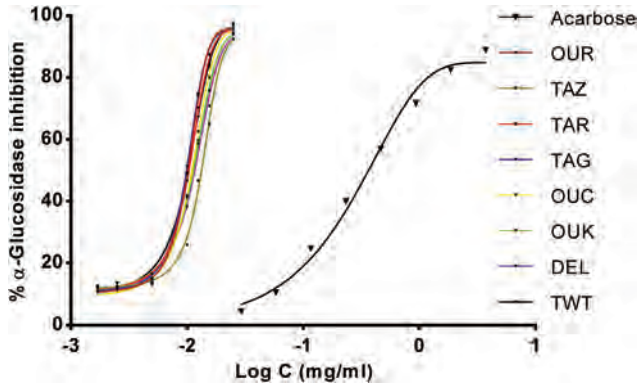

Fig. 3 Activity of date seeds on targets for type 2 diabetes. α-Glucosidase inhibition performed by DSE and acarbose.

α-GLU than acarbose (Table 5), with IC_{50} values ranging from 10.31 to 13.54 μg ml^{-1} whereas the IC_{50} value for acarbose was 369.38 μg ml^{-1}. However, most of our extracts did not show a prominent capacity to inhibit AChE or lipase in a wide range of concentrations; the highest inhibition percentage was observed only at a concentration of 1000 μg ml^{-1}. The results show that TAG and TAR cultivars are able to inhibit AChE (48.66%) and lipase (34.94%) activity, respectively (Fig. 4A and B). DSE inhibition activity was inferior compared to those of positive controls (galantamine and orlistat) in these bioassays.

The DSE did not exert activity against xanthine oxidase (XO) (data not shown).

3.5. Cytotoxicity screening in fibroblasts (3T3-L1 cell line)

The MTT assay was used to test whether DSE could affect 3T3-L1 cell viability. For this purpose, fibroblast cells were treated with a range of concentrations from 0.016 to 1 mg ml^{-1} for 24 h. All DSE showed very mild antiproliferative effects in 3T3-L1 cells (Fig. 5). Significant differences were detected at concentrations over 0.25 mg ml^{-1}, which indicates that this cell line seems to be partially sensitive to seed components. The cell viability was approximately 30% at the highest tested concentration (1 mg ml^{-1}); however, the mitochondrial activity was not significantly reduced at concentrations under 0.25 mg ml^{-1}, which can be considered as the physiological concentrations for bioactive substances.

4. Discussion

Date seeds are a potential source of valuable nutrients and phytochemicals and they could be considered as an inexpensive source of natural antioxidants.[39]

The results of chemical analysis on the date seed powder prepared from different cultivars showed appreciable amounts of ash and proteins. In general, the values of proteins were in agreement with those reported by previous studies.[19,39,40] The main differences were noted for the contents of ash and carbohydrates. In fact, Ahmed *et al.*[41] reported that date seeds of three varieties grown in different regions, Pakistan, Iran and Saudi Arabia, contained low amounts of ash, between 1.19 and 1.27%. In addition, the study published by Habib *et al.*[42] mentioned that the 18 different United Arab Emirates date seed

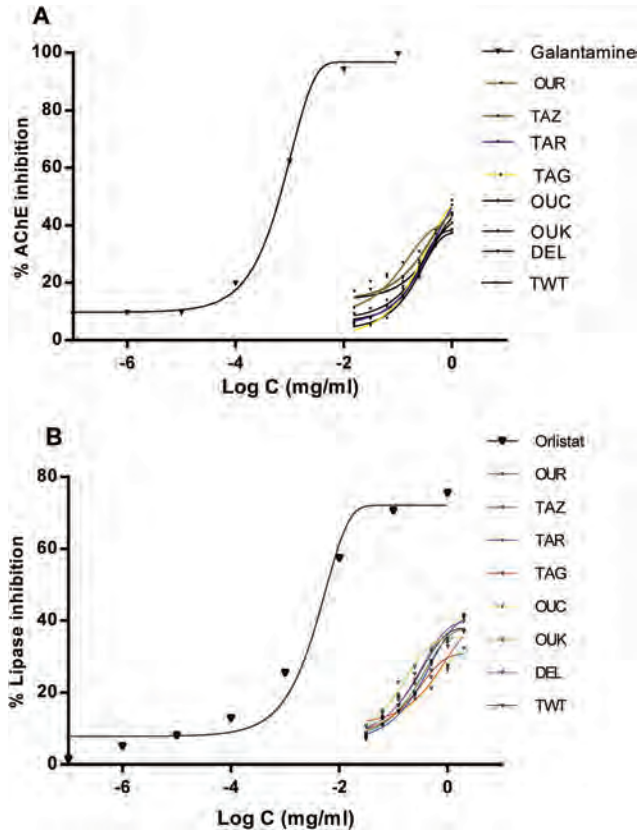

Fig. 4 Activity of DSE on targets for nervous and obesity system enzymes. AChE inhibition performed by different DSE and galantamine as a reference inhibitor (A). Lipase inhibition performed by date seeds with different concentrations, and orlistat as the standard (B).

cultivars contained 2.43 to 4.65% of carbohydrates, a value significantly lower than that observed in the present study.

The variations in nutrient compositions from instant findings may be attributed to various factors, basically the date fruit and both the normal variability of the cultivars and different environmental factors and farming practices.

In recent years, there has been growing interest in better investigation of not only the nutritional value of foods but also the health-beneficial properties. The importance of bioactive compounds in human health has been well recognized and the capacity to inhibit oxidative pathophysiological mechanisms could help prevent certain diseases.[1,4,8]

The phytochemical compounds described in our study may be involved in the numerous bioactivities that these extracts have shown. However, the phytochemical analysis of date by-products revealed high contents of TPC (475.51 mg GAE per g dw) and TFC (6.52 mg QE per g dw), which are higher concentrations compared to those from other studies.[43–45]

As reported in Table 2, quantitative analysis of the by-product extracts demonstrated that all of them contained notable AC, FC, PA and AAC. The best level was found in TAG cultivars. The identified phytochemical pattern is very much higher than those reported for date seeds in other investigations. The results of anthocyanin contents were higher than those reported by Metoui *et al.*[44] who found the highest value (0.6 mg CGE per g dw) in the Tunisian Deglet Nour variety. High concentrations of proanthocyanidins have been observed compared with the findings of El-Rahman *et al.*[46] and Ahmed *et al.*[47]

According to the HPLC-DAD-MS analysis, total phenolic compounds of lyophilized date seeds are in different proportions, highlighting the presence of three different phenolic acids and two flavonoids. These results demonstrated that *P. dactylifera* seeds used in this study may represent an interesting source of antioxidant compounds; however, other research studies show different identified phenolic compounds.[10,48,49] These differences may be due to the origin of the date fruit, cultivation and harvesting time, environmental factors related to date cultivars or phenolic compound quantification methods.

Since phytochemical compounds have the ability to scavenge free radicals, the antioxidant activity of the date by-product was determined using different antioxidant assays widely known as the ORAC, FRAP, DPPH, ABTS and superoxide radicals (xanthine/xanthine oxidase). Reviews of relevant literature studies revealed that other authors, who analyzed date seeds from other cultivars and regions, had previously reported slightly lower TEAC results than ours.[16] In this case, the comparison is difficult because each extract is prepared in a different way. The antioxidant capacity of date seeds from three cultivars namely Zahidi (Iran), Deglet Nour (Tunisia), and Khouat Allig (Tunisia) extracts was tested; the ORAC activity values were between 0.13 and 0.30 mmol TE per g fw.[50] On the other hand, date seeds showed values of ORAC activity of about 0.58 and 0.93 mmol T eq. per g fw,[39] which were lower than those of the seeds of eight cultivars used in this investigation.

Regarding the ferric reducing antioxidant power (FRAP), bioactive compounds can also act as metal chelators, preventing oxidation caused by highly reactive hydroxyl radicals.[51] According to this assay, seed extracts displayed the highest reducing power compared with Iranian seeds of fourteen cultivars.[52]

The DPPH radical is widely used in antioxidant studies.[53] In fact, DPPH$^{\bullet}$ free radical scavenging behavior of natural products is particularly dependent on the heterogeneous chemical structure and polarity of these phytochemicals. They can also have synergic and/or antagonistic mechanisms.[54,55] DSE has shown an ability to reduce both DPPH and ABTS radicals in a clearly dose dependent manner, with an IC_{50} of 37.30 to 68.90 and 13.98 to 32.31 μg ml^{-1}, respectively. A recent study with date seed methanolic extracts showed antioxidant activity in the same range with an IC_{50} of 41.30 μg ml^{-1},[56] while another report mentioned that the ABTS capacity for acetone extracts of two Tunisian date seed cultivars was very much lower than our finding with IC_{50} values of 820 and 1190 μg ml^{-1}.[57]

The fifth method used to evaluate the antioxidant properties of DSE is the xanthine/xanthine oxidase method, which

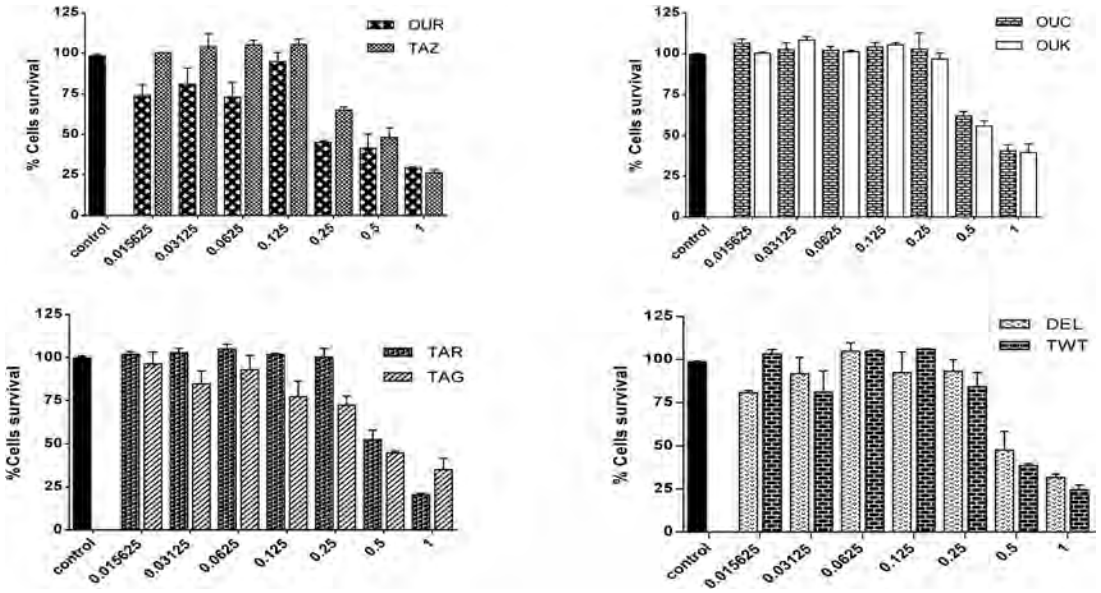

Fig. 5 Effects of eight different date seed cultivars on cell viability of 3T3-L1 measured by MTT assay: cytotoxicity after 24 h treatment with different concentrations of DSE from 0.016 to 1 mg ml^{-1}. Results are expressed as the percentage of cell viability over control cells (untreated cells).

is a more relevant method for generating free radicals from a physiological perspective as DPPH or ABTS are artificial radicals that do not exist in biological systems.[33] Furthermore, the DSE was not able to inhibit the XO enzyme; so we can conclude that the by-product acts in this method only by capturing the superoxide radical generated by the reaction of this enzyme. To the best of our knowledge, this is the first time that date seeds have been reported to scavenge superoxide radicals generated by the xanthine/xanthine oxidase system. All the tested extracts exhibited superoxide radical scavenging activity in a dose dependent manner, similar to other food plants.[58]

All these data indicate that seeds of the eight date cultivars displayed significantly different results on antioxidant activities; these differences may also be due to the presence of other bioactive compounds and also the action synergy of these components.

Finally, the study of the capacity of DSE to inhibit physiological enzymes was tested in order to detect potential applications as pharmaceutical products or food supplements.

Tyrosinase has been linked to skin disorders such as hyperpigmentation.[59] This enzyme is involved in tyrosine–melanin pigmentation *via* the production of dopaquinone derivatives and melanin induction. Additionally, some studies have demonstrated that the oxidized metabolites of dopamine known as dopamine quinone derivatives are thought to play a pivotal role in the degeneration of nigrostriatal dopaminergic neurons in Parkinson's disease.[60] In recent years, treatment possibilities with natural sources have gained importance and secondary metabolites from plant materials have been shown to exhibit tyrosinase inhibitory activity.[61] According to our data, these eight seed extracts may have potential as skin-whitening agents *via* TYR inhibition; however the DSE exerted a moderate level of AChE inhibition. On the other hand, our literature survey indicated that there are few reports on the TYR and AChE inhibitory effects of *P. dactylifera* seeds to date. However, different results were found in the study of Sekeroglu *et al.*[62] who reported that date seed extracts exhibit a strong inhibition against AChE.

Type 2 diabetes is a chronic metabolic disorder affecting millions of people worldwide. Its prevalence is on the rise globally at an alarming rate, which makes it one of the major growing health problems. Recently, the pressure to develop new drugs and substances for type 2 diabetes has been stimulated by the worldwide increase in the incidence of this disease.[63] One of the therapeutic strategies for effective control of postprandial hyperglycemia is to retard the hydrolysis of carbohydrates in order to slow down the intestinal absorption of glucose. Alpha-glucosidase inhibitors play a major role in the management of hyperglycemia by delaying the postprandial increase of the blood glucose level after a mixed carbohydrate diet.[64]

Moreover, it is remarkable that DSE exhibited strong inhibitory activity against α-glucosidase with IC_{50} values ranging from 10.31 to 13.54 μg ml^{-1} when compared to the positive control, acarbose (369.38 μg ml^{-1}). An important contribution towards α-glucosidase inhibition shown by the tested extracts may be made by the high level of ferulic acid. Previous studies have shown that phenolic compounds can also be used as neuroprotective agents, with therapeutic potential in hyperglycemia.[65,66] In fact, an *in vivo* study by Japanese researchers has demonstrated that the administration of ferulic acid to diabetic mice resulted in a decrease in blood glucose levels.[67] The efficacy of α-glucosidase inhibition by aqueous and methanolic extracts from date seed cultivars of different regions has been tested on glycemic control in

rats.[68,69] Interestingly, all tested extracts presented a better inhibitory effect than the results found by Khan *et al.*[11] and Thouri *et al.*[57]

For these reasons, DSE could be an interesting source of antioxidants and bioactive molecules to prevent metabolic disorders such as hyperglycemia.

Lipid metabolism is elegantly balanced to maintain homeostasis. When the balance is lost, obesity or hyperlipidemia develops, leading to a variety of serious diseases. One of the most important strategies in the treatment of obesity includes the development of inhibitors of nutrient digestion and absorption, in an attempt to reduce energy intake, and this can be remedied by inhibiting pancreatic lipase activity and by inhibiting or delaying lipid absorption.[70,71] Our findings from the lipase enzyme assay indicated that the DSE showed low lipase inhibition at a concentration of 1000 μg ml^{-1} (34.94% against 75.57% for the reference compound known as orlistat). Other authors such as Masmoudi-Allouche *et al.*[72] reported an important contribution of the Kentichi seed cultivar; our results are lower than those found for different date seed cultivars (IC_{50}: 1.21–186.4 μg ml^{-1}).

After previous assays on the antioxidant capacity, and neuroprotective, antidiabetic and antilipemic potential of different extracts of by-products, a question concerning safety issues of the DSE arose. Therefore, potential toxicity of freeze-dried date seeds affecting cell viability was assessed in murine fibroblast cells using crystal violet assay, which is based on the capacity of mitochondrial dehydrogenase enzymes of viable cells to covert the yellow tetrazolium salt MTT into a soluble dark blue formazan product. The cells were pretreated for 24 h with various concentrations of DSE ranging from 0.016 to 1 mg ml^{-1}. According to our findings, the reduction of 3T3-L1 cell viability determined as the mitochondrial activity was not observed at the lowest tested concentrations of DSE while the cell viability is affected at high concentrations (0.5 and 1 mg ml^{-1}), which represent high doses of this natural ingredient. Generally, it is observed that all extracts of different date by-products do not decrease the cell viability or produce a cytotoxic effect at a concentration below 0.25 mg ml^{-1}.

A number of researchers have stressed the need to study the possible toxicity of non-edible parts of the date fruit since few studies have shown certain toxicity of whole fruit extracts.[73] Other studies have reported better results in terms of antiproliferative or cytotoxic effects in cancer cell lines.[47,74] These differences may be explained based on the fact that many studies are performed with purified extracts.

5. Conclusion

In conclusion, this study provided new insights into the composition and biological activities of acetone extracts obtained from seeds of *Phoenix dactylifera* L. cultivars. Our findings provided evidence that all date seed cultivars form a valuable and interesting natural source of bioactive molecules with great antioxidant properties, and are potentially able to prevent neurodegenerative, metabolic or skin disorders. However, among the different cultivars, the results showed that TAG is the most interesting one in terms of the antioxidant content. Interestingly, our results showed that all date seed cultivars are effective inhibitors of tyrosinase and α-glucosidase. Finally, more studies must be completed for a better understanding of the mechanism of action of these extracts as antioxidants and/or pharmacological agents; it is necessary to analyze and identify the different active natural entities in order to understand the structure–activity relationship for each of the effects. These findings are very promising, allowing us to consider industrial exploitation and technological development in the food, pharmaceutical and cosmetic industries.

Conflicts of interest

The authors declare that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

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